MALARIA
MOSQUITO ENGINEERING

Building a Disease-Fighting Mosquito

In a futuristic scheme, researchers are trying to engineer a malaria-resistant mosquito to replace natural populations

She lands on your arm so softly she doesn't even wake you up; neither do her frantic attempts to find a blood vessel during the next minute and a half. Repeatedly, she thrusts her needle-sharp, double-barreled mouthpiece into your skin, trying her luck. As she sucks through one barrel to see if anything comes up, she spits a sophisticated mix of drugs down the other to prevent blood from clotting and your vessels from constricting. When she finally hits a vein, she quickly fills up her gut. Then she leaves. One meal is enough: As she flies away, suddenly three times heavier, she has enough proteins on board to produce about 100 eggs a few days from now.

Meet the female *Anopheles* mosquito, arguably the most dangerous animal in the world. It's not those 3 or 4 microliters of blood that she steals; it's the sinister gift that she sometimes leaves behind, some of the *Plasmodium* parasites that cause malaria. Within weeks you may come down with a fever; if you're a child or a pregnant woman, the encounter may kill you, as it does 1 million or more people every year.

For decades, scientists have tried to stop her from spreading disease. And now, with resistance against insecticides on the rise and a U.N.-backed push to phase out DDT, several labs have embarked on the most ambitious and futuristic of all approaches to combat malaria: They hope to replace billions and billions of mosquitoes in the world's endemic areas with new strains, created in the lab, that would be "refractory," or unable to transmit the parasite.

The idea is not that farfetched, these researchers claim. In a paper scheduled for publication in the *American Journal of Tropical Medicine and Hygiene*, for instance, a team led by Anthony James of the University of California, Irvine (UCI), reports that it has created mosquitoes that produce antibodies against *Plasmodium*. In lab studies, these antibodies reduced the number of parasites in the insects' salivary glands—their last stop in the mosquito's body—by 99.9%.

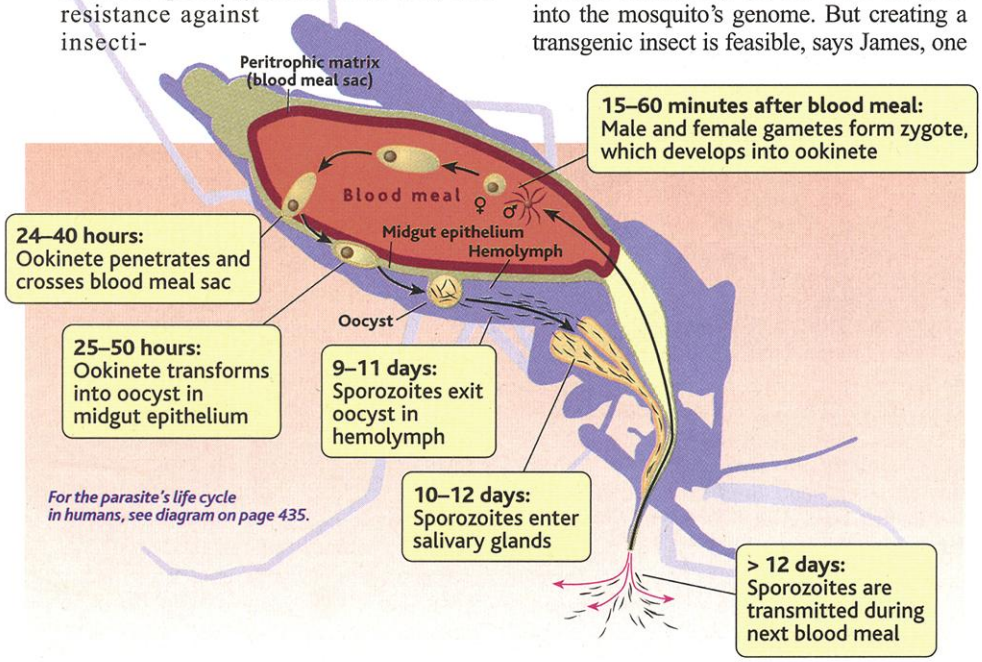
Granted, says James, the researchers used *Plasmodium gallinacium*, which infects chickens, not humans, and its mosquito host, *Aedes aegypti*, rather than the feared *Anopheles*. And they cut corners by infecting the mosquitoes with a smartly designed virus that expresses the antibody gene rather than creating a true transgenic—in other words, they did not insert the gene into the mosquito's genome. But creating a transgenic insect is feasible, says James, one of several researchers trying to reach that goal. Indeed, most researchers optimistically predict that the first malaria-resistant *Anopheles* larvae will crawl out of their eggs in a lab somewhere within a few years.

Others agree the study is an important step. "It's the first time anybody has shown you can actually make a mosquito refractory to a malaria parasite," says Joe Vinetz of the University of Texas Medical Branch (UTMB) in Galveston. "I think it's really neat."

It's also a welcome boost for a field that still faces enormous hurdles. To realize the grandiose plan of building a "better mosquito," researchers have to overcome three problems. First, they have to find genes that frustrate the parasite's life cycle, complete with switches to turn them on in the right place and time in the mosquito's body. Next, they need a way to stick those genes into the mosquito. Finally, they have to devise a way to give their winged creation a flying start in the real world, so that it can beat out natural populations—not in a centuries-long, evolutionary struggle, but in a few years.

The second step—finding a way to genetically engineer mosquitoes—has long stymied researchers. In the 1980s, they had hopes of borrowing a technique used for years to equip the fruit fly, *Drosophila*, with new genes. In the fly, researchers simply attach the desired gene to a so-called transposon, a short piece of DNA that knows how to worm itself into the fly's genome. Then they inject the transposon into a fly egg; with luck, it lands somewhere on a chromosome where it works and doesn't harm other genes. But however hard they tried, researchers couldn't get the standard *Drosophila* transposon, simply called *P*, to deliver a gene—any gene—in mosquitoes. "It put a cloud over the whole research area," says James. "At meetings, we were always talking about things that didn't work."

That started to change in the 1990s with the discovery of a series of new transposons, such as *Hermes*, a house fly transposon discovered by David O'Brochta of the University of Maryland, College Park, and Peter Atkinson of the University of California, Riverside. In 1998, James's team, collaborating with Frank Collins (who is now at the University of Notre Dame in Indiana) and others at the Centers for Disease Control and Prevention (CDC) in Atlanta, used *Hermes* to insert an eye color gene in *Aedes aegypti*. And last June, Andrea Crisanti's team at the European Molecular Biology Laboratory in Heidelberg, Germany, reported slipping the gene for a green fluorescent protein into *Anopheles stephensi*, the mosquito that transmits malaria in India, with yet another transposon called *Minos*. Now, several research groups are feverishly trying to tinker with the



Achilles' heels. Each stage of *Plasmodium*'s life cycle presents opportunities to thwart the parasite.

ILLUSTRATION: A. STONEBRAKER

biggest killer of all: *Anopheles gambiae*, the main malaria vector in Africa. Success is just months away, predicts CDC's Mark Benedict.

Meanwhile, other researchers have been making considerable progress on the first step: finding genes that can thwart the parasite. In theory, every stage of *Plasmodium*'s life cycle in the mosquito is fair game. After being sucked up during a blood meal, *Plasmodium*'s male and female gametes merge in the blood droplet inside the mosquito's gut. Eventually, they form a so-called ookinete, which pierces the sturdy sac surrounding the blood meal and nestles inside the gut wall. There, the ookinete develops into an oocyst, which eventually bursts open on the other side of the gut, releasing thousands of minuscule sporozoites into the mosquito's circulatory system, which is filled with a fluid called hemolymph. The sporozoites travel to the salivary gland, force its wall open, and take up residence in the mosquito's saliva—ready for the next round in humans.

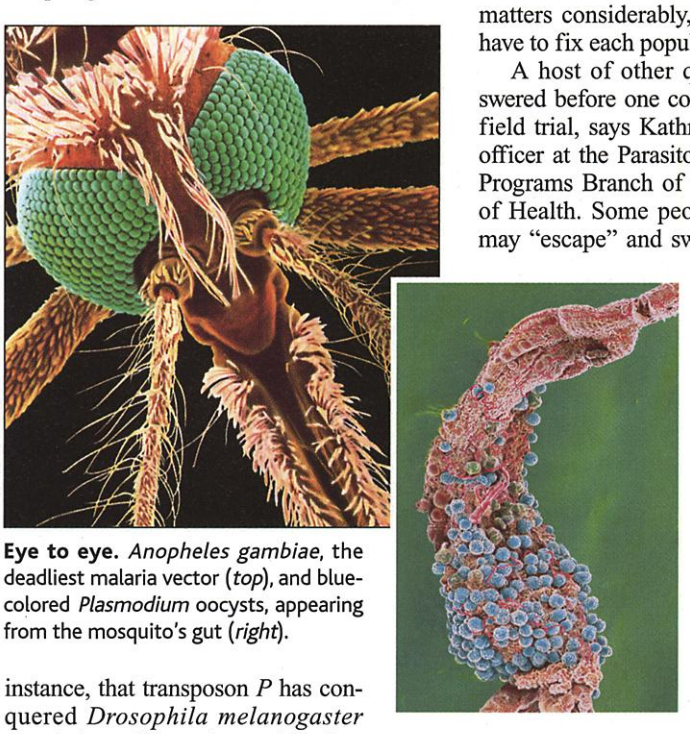
Alexander Raikhel and colleagues at Michigan State University in East Lansing want to attack the sporozoites as they float in the hemolymph. To do so, they recently took the gene for defensin, an antimicrobial compound that occurs naturally in *Aedes* mosquitoes, and coupled it to a promoter—a stretch of DNA that determines when and where a gene is switched on—that activates the gene only after the mosquito has eaten. When they built this ensemble into *Aedes* mosquitoes, the researchers reported 2 months ago, it boosted defensin levels in the mosquito's hemolymph. They're currently testing whether this also drives down *P. gallinacium* sporozoite numbers.

UCI's James also attacks sporozoites in *Aedes* mosquitoes, but with a different strategy. As they describe in their upcoming paper, the researchers first coaxed mouse cells to produce antibodies against CSP, a protein known to occur on the sporozoites' outer coat. Next they created an artificial gene coding for a slightly altered version of that antibody and used a virus to infect the mosquitoes with the construct. The insects started producing antibodies, which apparently attack the sporozoites very efficiently, leading to the 99.9% decrease in the salivary glands. James is now trying to do the same in *Anopheles*.

UTMB's Vinetz, meanwhile, is eyeing a much earlier stage of the parasite's life cycle—its piercing of the blood meal sac—using a *Plasmodium* enzyme he discovered last year.

With so many options, researchers are confident they'll be able to engineer a refractory *Anopheles gambiae* one way or the other. But step three—replacing existing mosquito populations with that humanmade critter—is the real sticking point, says Marcelo Jacobs-Lorena of Case Western Reserve University in Cleveland. "That's not going to be easy at all."

Many mosquito engineers hope the very same transposons they use to slip a gene into the mosquito's chromosome can also perform this trick. That's because some transposons have a thoroughly selfish way of spreading: Whenever two individuals mate, one of which has the transposon, all of their offspring inherit it. Researchers know, for instance, that transposon *P* has conquered *Drosophila melanogaster* populations all over the world in just the past century.



Eye to eye. *Anopheles gambiae*, the deadliest malaria vector (*top*), and blue-colored *Plasmodium* oocysts, appearing from the mosquito's gut (*right*).

But researchers have no idea whether *Hermes*, *Minos*, and other transposons used to transform mosquitoes today could have the same effect. Nor do they know if a transposon and an artificially attached gene would stay together as the transposon started spreading.

Others are banking on a different way to convert the mosquito population: by enlisting *Wolbachia*, a bizarre bacterium that lives inside the cells of many insects. To favor its own reproduction, *Wolbachia* ensures that whenever an infected and an uninfected partner mate, either all their offspring will be infected or there will be no offspring at all. As a result, *Wolbachia* can spread like wildfire.

If you could stick some of the genes that can make mosquitoes refractory into *Wolbachia*, then infect mosquitoes with the bug and set them loose in a natural population, you might make it refractory in a relatively short time, speculates Scott O'Neill, a *Wolbachia* researcher at Yale University. The catch is that researchers don't know of any *Wolbachia* species that naturally inhabit *Anopheles*, but they could probably figure out a way to trick one into doing so, says O'Neill.

But even if these dreams come true, more problems loom. Entomologists don't know all that much about *Anopheles* populations, but recent studies by UTMB's Gregory Lanzaro and his colleagues in Mali have shown that what looks like one *Anopheles gambiae* population is in fact several different subpopulations that don't interbreed much. That could complicate matters considerably, as researchers would have to fix each population separately.

A host of other questions must be answered before one could even think about a field trial, says Kathryn Aultman, program officer at the Parasitology and International Programs Branch of the National Institutes of Health. Some people think transposons may "escape" and sweep through other insect populations, with unknown effects. The first field trial would probably be held on an island to contain the transposon, says Aultman. "If it turns out to have been a disastrously terrible idea, you can just spray the place down with malathion," she says.

Further complicating matters, it appears that each transposon can only sweep through a population once. If so, researchers would have to choose the attached gene very judiciously, lest they blow their only chance. And finally, the idea of releasing transgenic mosquitoes may prove highly controversial, if the recent backlash against transgenic crops is any indication. "We're planning some meetings to start discussing all of these issues," says Aultman. With so many uncertainties, spending money on a vaccine or new drugs may seem a safer bet. But James defends this unconventional approach: "The malaria vaccine has been predicted to be just around the corner for at least 2 decades. I think spending a few percent on something else is a reasonable investment." Fortunately, adds Vinetz, researchers would not have to wipe out each and every natural mosquito. Models have shown that even when only part of the population becomes refractory, transmission may drop enough for the epidemic to peter out.

If they're right, mosquitoes will still keep hunting for your blood, as they have for at least 150 million years. But there's a chance they will leave you with just an itch and not a malaria parasite. **–MARTIN ENSERINK**

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